AD_____

Award Number: W81XWH-10-2-0091

TITLE: Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress

PRINCIPAL INVESTIGATOR: Dr. Raymond Genovese

CONTRACTING ORGANIZATION: The Geneva Foundation
Lakewood, WA 96499

REPORT DATE: September 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-09-2011	Annual	1 SEP 2010 - 31 AUG 2011

4. TITLE AND SUBTITLE
Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-2-0091

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Raymond Genovese

E-Mail: RAYMOND.GENOVESE@US.ARMY.MIL

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Geneva Foundation
Lakewood, WA 96499

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) are major medical issues for the warfighter. The current project is designed to evaluate the impact of mild traumatic brain injury (using blast over pressure) and traumatic stress (using a predator exposure procedure and conditioned fear procedure) in a rodent model. The studies evaluate these insults alone and in combination to specifically address the question of whether mTBI can exacerbate the effects of psychological stress. Additionally, following the insults, a molecular biological evaluation is performed based upon the discovery of biomarkers that have been shown to be correlated with other forms of TBI. Thus, the project aims to systematically assess the combined effects of blast overpressure, traumatic stress and learned stress responses in rodents with the aim of understanding how these forces may interact to impact behavior as well as evaluating their outcome on known biomarkers involved in TBI and stress response system activation. This project is a new start and while progressing, results are too incomplete to provide conclusions at this point.

15. SUBJECT TERMS
Traumatic brain injury, post-traumatic stress disorder, blast over pressure

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	19	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION:

Mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) are major medical issues for the warfighter. Often times, mTBI and PTSD present a convergence of symptoms, making it difficult to distinguish between the behavioral manifestations of the two conditions and to determine the extent to which the processes of traumatic stress and mild brain injury may synergize. The current project is designed to evaluate the impact of these two insults in a rodent model. To model the effects of mTBI, we are using blast over pressure (BOP). Two procedures are used to model traumatic stress / PTSD; first a predator exposure procedure is used to present a traumatic stressor to the rat; second, a conditioned fear procedure is used to model a process known to be disrupted in PTSD. Most notable, the studies evaluate these insults alone and in combination to specifically address the question of whether mTBI can exacerbate the effects of psychological stress. The studies are focused on the evaluation of short- and long-term behavioral impact from the insults, and use dependent measures from procedures including operant performance, conditioned suppression (conditioned fear), Morris water-maze and elevated plus maze. Additionally, following the insults, a molecular biological evaluation is performed based upon the discovery of biomarkers that have been shown to be correlated with other forms of TBI. Thus, the project aims to systematically assess the combined effects of blast overpressure, traumatic stress and learned stress responses in rodents with the aim of understanding how these forces may interact to impact behavior as well as evaluating their outcome on known biomarkers involved in TBI and stress response system activation.

BODY:

The project award date was 01 Sep 10. A three-way cooperative research and development agreement (CRADA) between the Army (WRAIR), the Navy (NMRC) and the Geneva foundation was negotiated and approved on 01MAR11. The CRADA was a required step before work on the study could progress.

The project is broken up into three tasks, with a number of subtasks under Task 2 and Task 3.

Task 1: Generation of approved IACUC protocols. We have generated one protocol and gained WRAIR/NMRC approval. The ACURO oversight body has also approved the protocol. The appendix contains approval letters for the local IACUC and the ACURO. A second protocol is still being prepared. Essentially, the approved protocol covers work in Task 3, which is well underway. The second protocol covers studies for Task 2.

Task 2: Evaluation of combination BOP and predator exposure on Morris water maze and elevated plus maze with subsequent biomarker assay (total number of rats=80). Work on this Task has not yet started.

Task 3: Characterization of BOP on Conditioned Fear with subsequent biomarker evaluation (total number of rats=60). Progress on this Task is encouraging and is slightly

ahead of schedule.

Subtask 1: Acclimation / food restriction. We have completed this subtask for 28 rats.

Subtask 2: VI32 Acquisition. We have completed this task for 12 rats. An additional 16 rats are currently in this phase of the study. Figure 1 shows the VI32 acquisition for a representative rat. Following subtask 1, subjects are shaped to lever press for food and then moved to the VI32 schedule (e.g., day 1 on the graph). Sessions are then conducted daily (Mon-Fri) until performance is stable as evidenced by visual inspection of cumulative response records and evaluation of deviation from the moving average and/or daily fluctuation range in response rate. We estimated 2-3 months for this training. In some cases it will be somewhat longer and in others, somewhat shorter.

Subtask 3: BOP / Inescapable Electric Shock (IES) treatments. We have completed this task for 12 rats. No particular issues were encountered.

Subtask 4: CER evaluations. We have completed this task for 12 rats. For Study 2A (in this task), CER evaluations take place over two months. Performance data for a representative rat over that period of time is illustrated in Figure 6. For study 2B, a single CER test is administered shortly after the last BOP administration. Figure 7 illustrates performance on subtask 4 for a representative subject in 2B.

We have completed long-term CER evaluations (2A) for 8 rats, n=2 for each treatment. Graphic illustration of the CER evaluations as first-press time and as a suppression index for each group appear in Figures 2-5 (Figure 2, Sham IES + Sham BOP; Figure 3, IES + Sham BOP; Figure 4, Sham IES + BOP; Figure 5, IES + BOP). We have completed short-term CER evaluations (2B) for 4 rats. The CER evaluations for the latter rats (as a suppression index) are shown in Figure 8.

Subtask 5: Tissue harvesting and proteomic analysis. We have completed tissue harvesting from 12 rats. Most unfortunately, all tissue samples for 8 rats (all from 2A) were destroyed during a power outage over a weekend. The incident was thoroughly investigated (samples from other studies were also lost) and the problem has been remedied. Proteomic analysis of the tissue samples from 4 other rats (2B) is in progress.

KEY RESEARCH ACCOMPLISHMENTS:

- Gained WRAIR/NRMC and ACURO approval for animal use protocol.
- Implemented VI32 and Conditioned Fear procedures.
- Implemented mild TBI blast procedure.

REPORTABLE OUTCOMES

Genovese, RF and Ahlers, S. Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress. Department of Defense (DOD) Traumatic Brain Injury (TBI) Biomarkers In-Progress Review, 13 & 14 August 2011, Ft. Lauderdale, FL

CONCLUSION:

As this project is in its infancy, we simply cannot draw conclusions based on such a small sample of results. Nevertheless, we can describe the results in regard to some speculative "trends".

Most striking is that rats receiving IES (in the fear conditioning procedure) and BOP (75kPa X 3) do not exhibit a CER as those in normal fear conditioning. Figure 3 shows the CER in rats receiving IES and sham BOP. The CER is evident by the large first press time (top) and large suppression index (bottom) during the first two weekly test sessions. Thereafter, extinction (or more accurately, learned safety) occurs and the values for both measures become similar to those seen during all test sessions in groups not receiving IES (e.g., see Figure 2). This result is typical of a normal fear conditioning paradigm. Figure 5 shows the results for the rats receiving the IES and also the BOP. No evidence of fear conditioning is observed in those rats. While we had hypothesized that BOP might enhance fear conditioning, the opposite appears to be suggested based on this small sample. Furthermore, in the single rat receiving IES and BOP for the short-term CER evaluation (2B), the suppression index value is much less than for the IES + sham blast rat (see Figure 8). It may be that the BOP produces retrograde amnesia affecting the preceding conditioning (IES +light/tone). It will be interesting to see how this trend endures as we complete subjects in short-term and long-term conditions of this task.

We see no evidence of a dramatic impact of BOP on VI32 performance – either short-term (see Figure 7) or long-term (see Figure 6).

We were somewhat concerned that the isoflurane administration (used in BOP and sham BOP treatments) might affect performance on the VI32, or even the fear conditioning – this does not appear to be the case thus far as sham BOP treatments do not appear to be disrupting performance.

It is too early in the study to offer further speculations.

REFERENCES:

None.

APPENDICES:

A1. Acronym Definitions

BOP: blast overpressure. In our procedure, we are using three exposures at 75 kPa (~11 psi).

CER: conditioned emotional response. With regard to the conditioned fear procedure, it refers to the conditioned response.

CS: Conditioned Stimulus. With regard to the conditioned fear procedure, it refers to the flashing lights and pulsing tone stimuli paired with IES initially and subsequently presented alone in the VI32.

IES: inescapable electric shock. In our procedure, the CS is paired with the IES to produce the fear conditioning.

mTBI: Mild traumatic brain injury. In our project this is implemented through the BOP.

SI or suppression index. A measure to evaluate the degree of response suppression on the conditioned fear procedure, i.e., a measure of the magnitude of the CER. Calculated by the formula (response rate before-response rate after) / (response rate before + response rate after). A suppression index is usually calculated for 1-, 2- and 3-min intervals before and after presentation of the CS.

VI32: Variable interval 32 second schedule of reinforcement. The operant conditioning schedule specifying that one lever press following an average interval of 32 sec produces reinforcement. Individual intervals are normally distributed around a mean of 32 seconds.

A2. IACUC Approval

DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
503 ROBERT GRANT AVENUE
SILVER SPRING, MD 20910-7500

REPLY TO
ATTENTION OF

MCMR-UWN

24 January 2011

MEMORANDUM FOR Raymond F. Genovese, Ph.D., Department of Behavioral Biology, Division of Psychiatry and Neurosciences, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500

SUBJECT: Protocol Approval, WRAIR/NMRC Institutional Animal Care and Use Committee

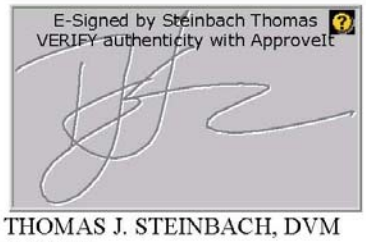
1. Protocol 11-PN-14, Entitled: "Neurocognitive and biomarker evaluation of combination mTBI from blast overpressure and conditioned fear," is approved by the WRAIR/NMRC IACUC for 60 rats. The protocol will expire 23 January 2014.

2. So that the protocol remains active and in compliance with all regulations, you will be required to complete an Annual Review Form for each year that the protocol is active. The IACUC will review the protocol annually, to comply with Federal Law.

3. The PI is required to submit an amendment requesting a change of PI if he or she is being transferred or leaves the Institute for any reason. Active protocols must be transferred to a new PI prior to the departure of the original PI. In addition, the original PI must submit a progress report (Annual Review) covering any work done in the current fiscal year prior to the transfer. Out-processing personnel will not be able to clear the ACUP office until these requirements have been met.

4. Before acquiring any animals, a protocol-planning meeting is highly recommended to ensure that issues such as animal housing and monitoring, acquisition of special supplies/equipment, occupational health, and safety are addressed. Such meetings should include the PIs, associate investigators, technicians, and supporting veterinary and animal care staff, see Protocol Planning Meeting Guide. More information is available on the Animal Care and Use Program web site.

5. POC for this action is Mr. Seitu Q. Khafre, 301-319-9051, or the undersigned, 301-319-7490.



THOMAS J. STEINBACH, DVM
LTC, VC
Chair, WRAIR/NMRC Institutional Animal Care and Use Committee

CF:
C, DAM
DAH
Consulting Vet, LTC Stephens-DeValle
IACUC Member
DIV Director

A3. ACURO Approval

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MD 21702-5012

REPLY TO
ATTENTION OF

January 26, 2011

Director, Office of Research Protections
Animal Care and Use Review Office

Subject: Review of USAMRMC Proposal Number PT090121, Award Number W81XWH-10-2-0091 entitled, "Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress"

Principal Investigator Raymond Genovese
Walter Reed Army Institute of Research (WRAIR), Silver Spring
Silver Spring, MD

Dear Dr. Genovese:

Reference: (a) DOD Instruction 3216.01, "Use of Animals in DOD Programs"
(b) US Army Regulation 40-33, "The Care and Use of Laboratory Animals in DOD Programs"
(c) Animal Welfare Regulations (CFR Title 9, Chapter 1, Subchapter A, Parts 1-3)

In accordance with the above references, protocol PT090121 entitled, "Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Conditioned Fear," IACUC Protocol Number 11-PN-14 is approved by the USAMRMC Animal Care and Use Review Office (ACURO) for the use of rats and will remain so until its modification, expiration or cancellation. This protocol was approved by the Walter Reed Army Institute of Research (WRAIR), Silver Spring IACUC.

When updates or changes occur, documentation of the following actions or events must be forwarded immediately to ACURO:

- IACUC-approved modifications, suspensions, and triennial reviews of the protocol (All amendments or modifications to previously authorized animal studies must be reviewed and approved by the ACURO prior to initiation.)
- USDA annual program/facility inspection reports
- Reports to OLAW involving this protocol regarding

  a. any serious or continuing noncompliance with the PHS Policy;
  b. any serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals; or
  c. any suspension of this activity by the IACUC

- USDA or OLAW regulatory noncompliance evaluations of the animal facility or program
- AAALAC, International status change (gain or loss of accreditation only)

Throughout the life of the award, the awardee is required to submit animal usage data for inclusion in the DOD Annual Report on Animal Use. Please ensure that the following animal usage information is maintained for submission:

- Species used (must be approved by this office)
- Number of each species used
- USDA Pain Category for all animals used

For further assistance, please contact the Director, Animal Care and Use Review Office at (301) 619-2283, FAX (301) 619-4165, or via e-mail: acuro@amedd.army.mil.

Sincerely,

For 

Alec Hail, DVM, DACLAM
Colonel, US Army
Director, Animal Care and Use
Review Office

Copies Furnished:
Mr. Christopher L. Baker, US Army Medical Research Acquisition Activity (USAMRAA)
Dr. Bao-Han Vu, CDMRP
Mr. Seitu Q. Khafre, Walter Reed Army Institute of Research (WRAIR), Silver Spring
Ms. Terri Western, Walter Reed Army Institute of Research (WRAIR), Silver Spring

SUPPORTING DATA: Figures 1-8.

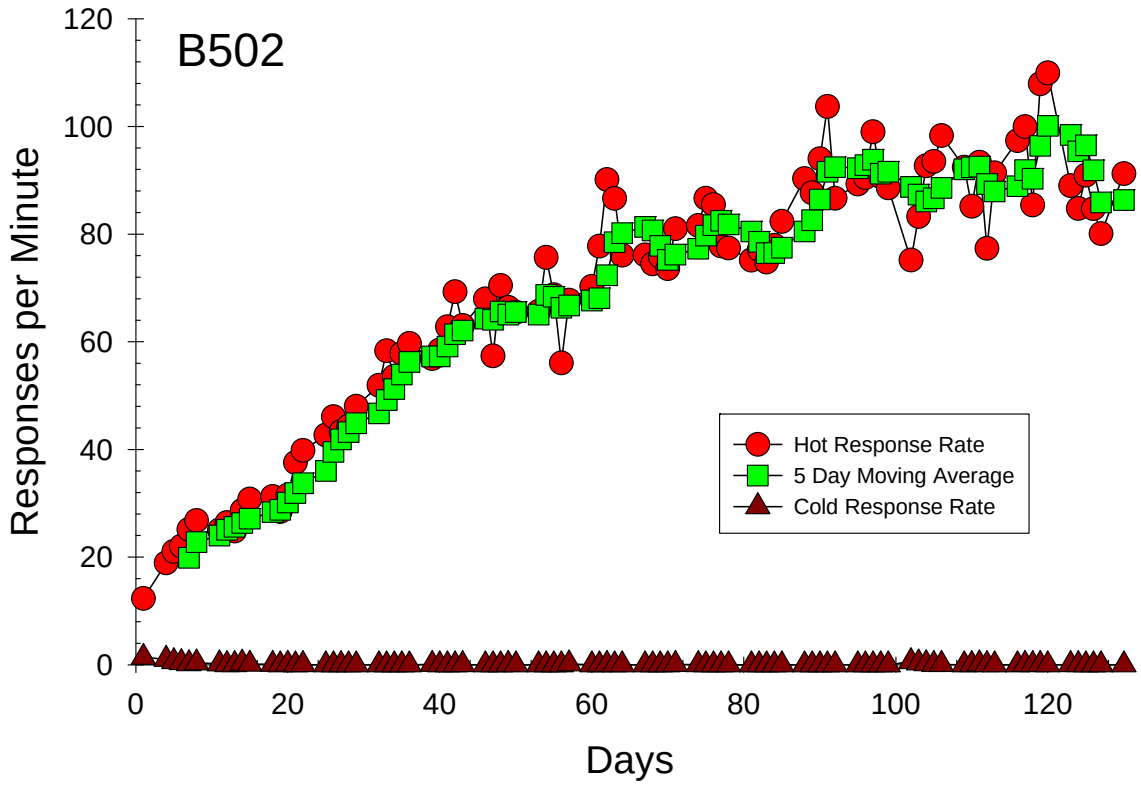


Figure 1. Acquisition of stable responding in a single rat on the VI32 schedule. Ordinate: responses (lever presses) per minute. Abscissa: consecutive days. Day 1 represents the first day that the VI32 was conducted. Circles represent session response rates on the active lever (the lever associated with food reinforcement, "Hot lever"). Squares represent the five day moving average for responding on the hot lever. Triangles represent rate of responding on a second lever that is never associated with food delivery ("Cold lever").

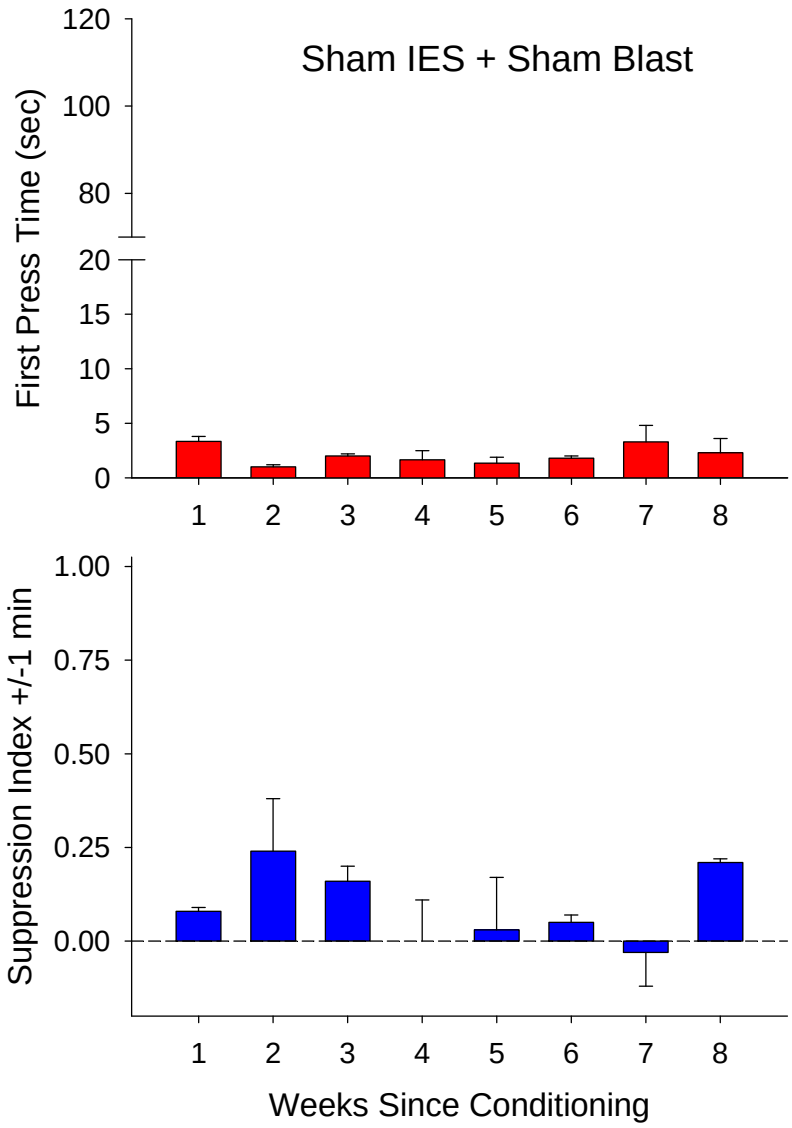


Figure 2. Fear conditioning performance in rats over 8 weekly test sessions. Each bar represents data from two rats receiving sham IES and three sham blast overpressure exposures. Abscissa: Weeks since fear conditioning. Ordinates: First Press time (top panel) and 1-min suppression index (bottom panel) from CER tests embedded in the VI32 session.

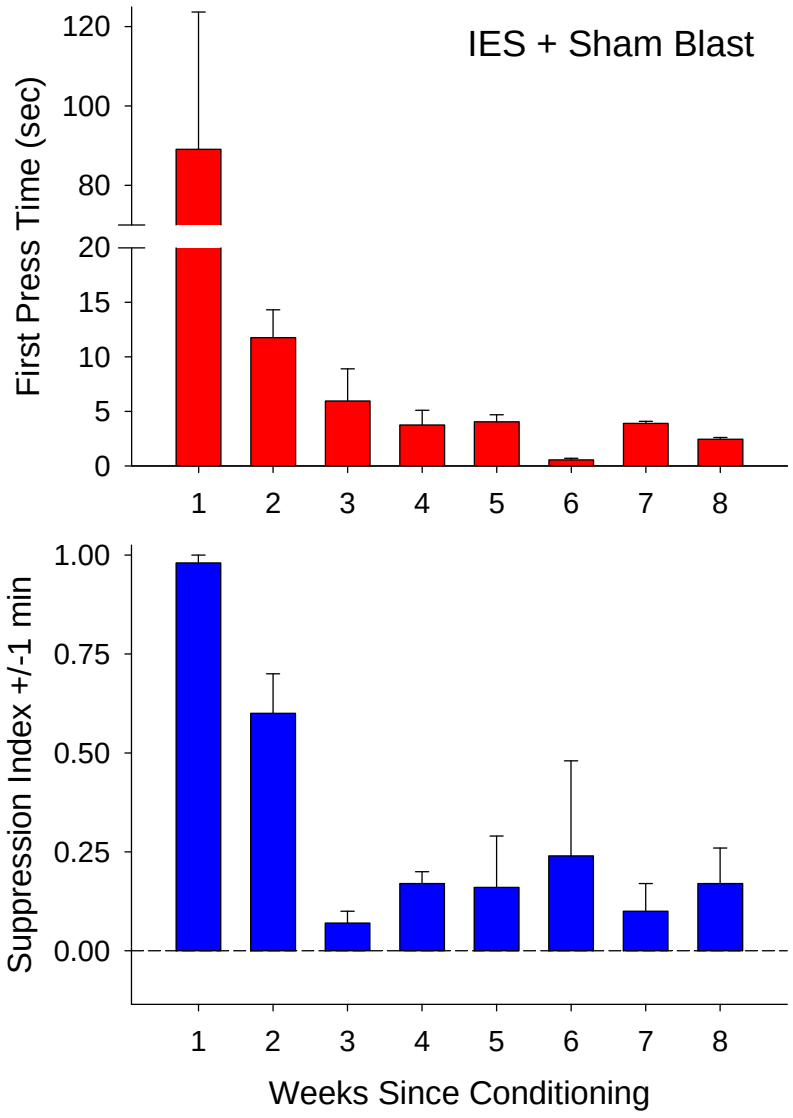


Figure 3. Fear conditioning in rats over 8 weekly test sessions. Each bar represents data from two rats receiving IES and three sham blast overpressure exposures. Abscissa: Weeks since fear conditioning. Ordinates: First Press time (top panel) and 1-min suppression index (bottom panel) from CER tests embedded in the VI32 session.

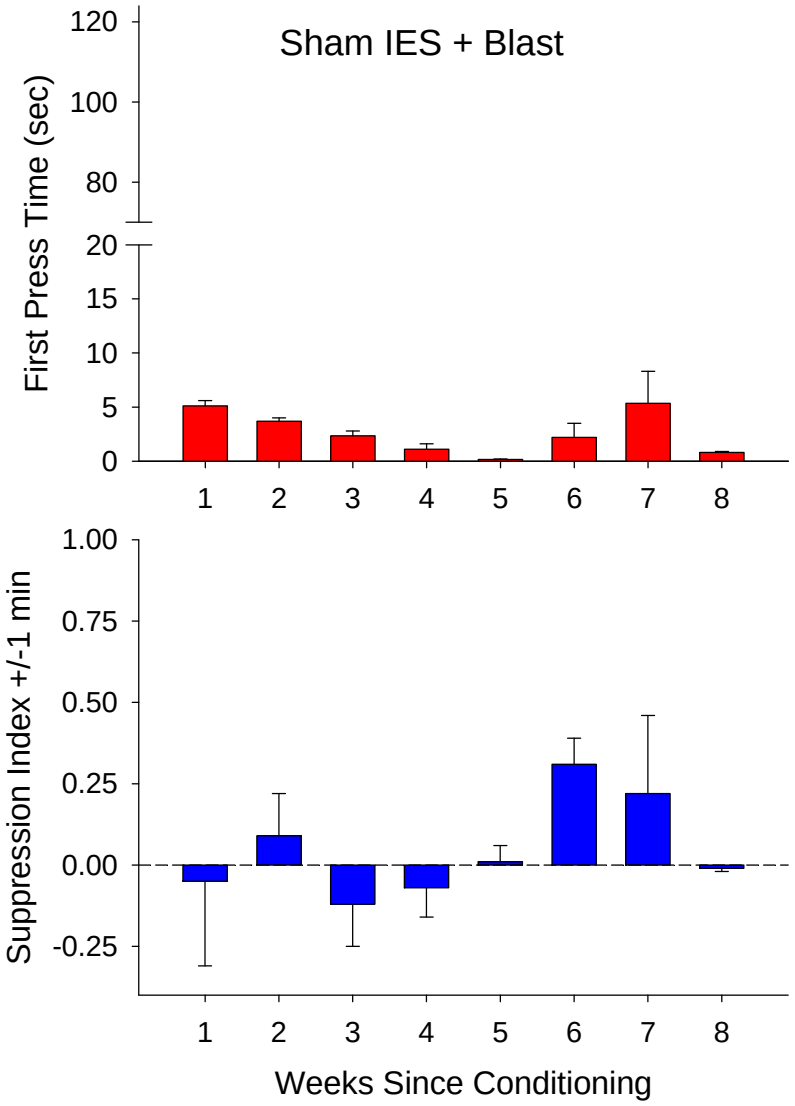


Figure 4. Fear conditioning in rats over 8 weekly test sessions. Each bar represents data from two rats receiving sham IES and three blast overpressure exposures (75 kPa). Abscissa: Weeks since fear conditioning. Ordinates: First Press time (top panel) and one min suppression index (bottom panel) from eight CER tests embedded in the VI32 session.

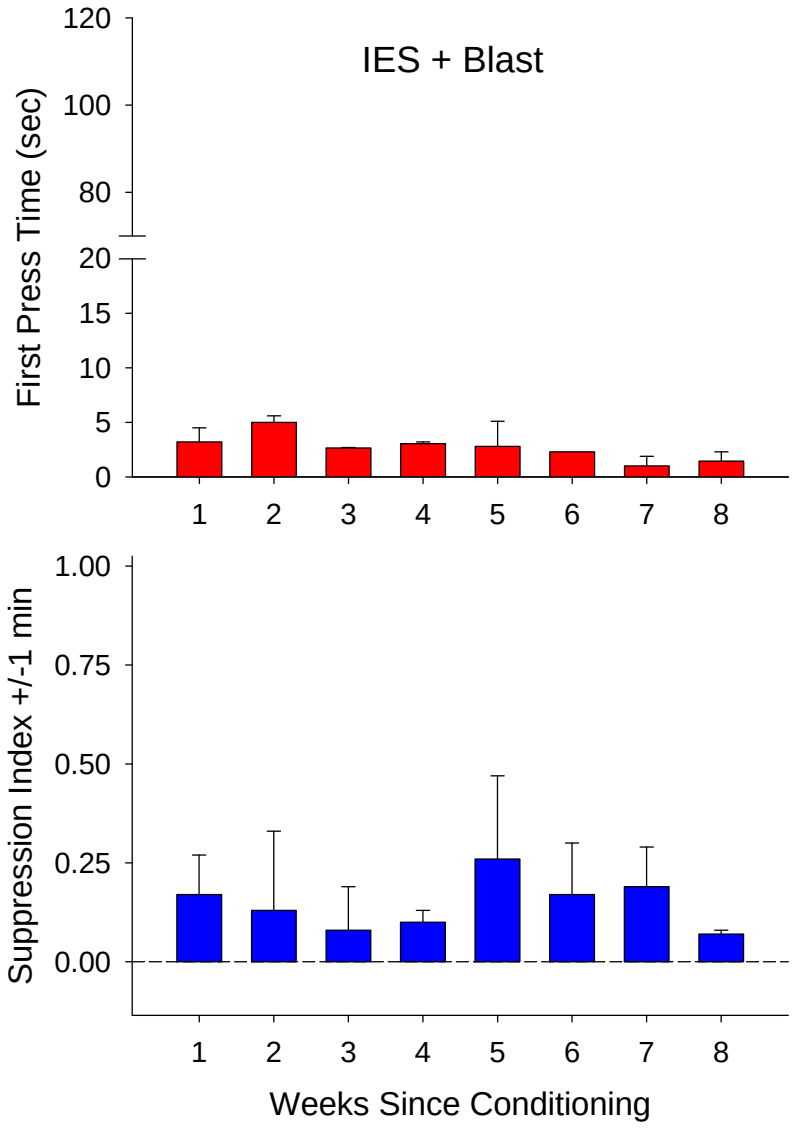


Figure 5. Fear conditioning in rats over 8 test sessions. Each bar represents data from two rats receiving IES and three blast overpressure exposures (75 kPa). Abscissa: Weeks since fear conditioning. Ordinates: First Press time (top panel) and 1-min suppression index (bottom panel) from eight CER tests embedded in the VI32 session.

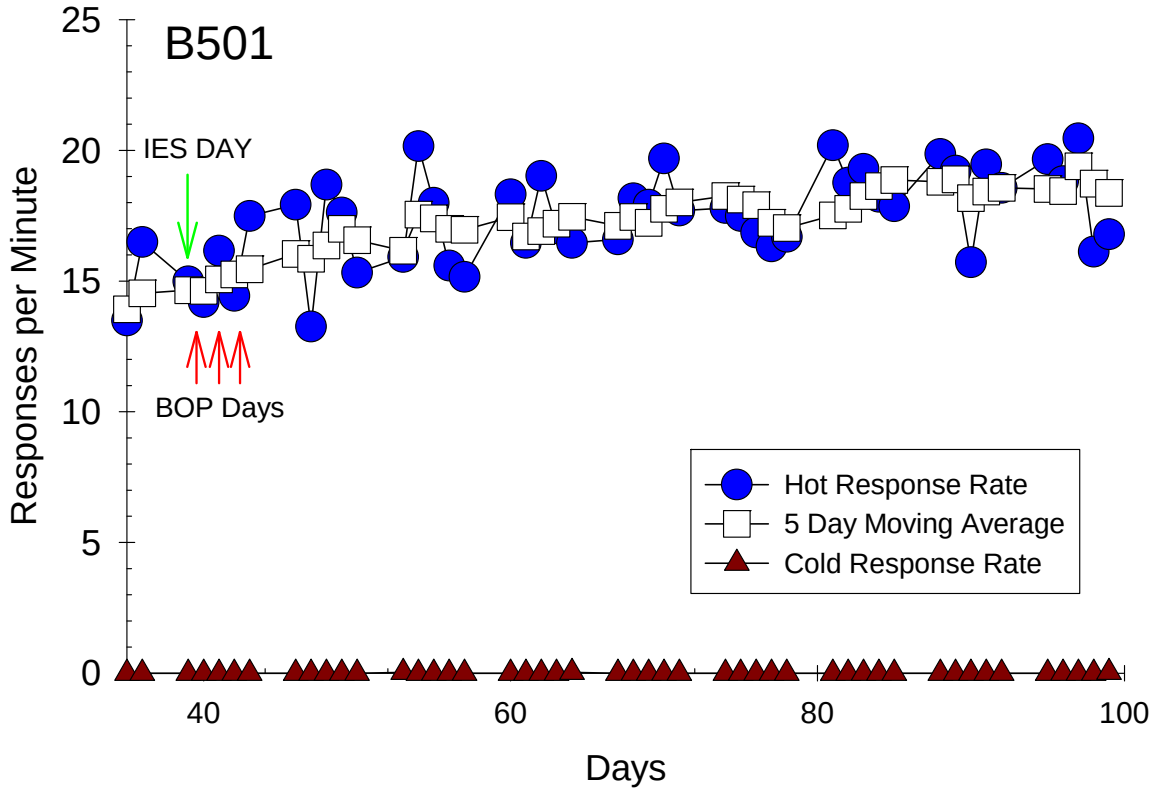


Figure 6. Performance of a single rat on the VI32 schedule. Ordinate: responses (lever presses) per minute. Abscissa: consecutive days. Circles represent session response rates on the active lever (the lever associated with food reinforcement, "Hot lever"). Squares represent the five day moving average for responding on the hot lever. Triangles represent rate of responding on a second lever that is never associated with food delivery ("Cold lever"). IES indicates day of the offline pairing of inescable electric shock and audio-visual stimuli (~ two hours after the session). BOP denotes days when blast overpressure was presented, 75kPa, (~two hours before the session).

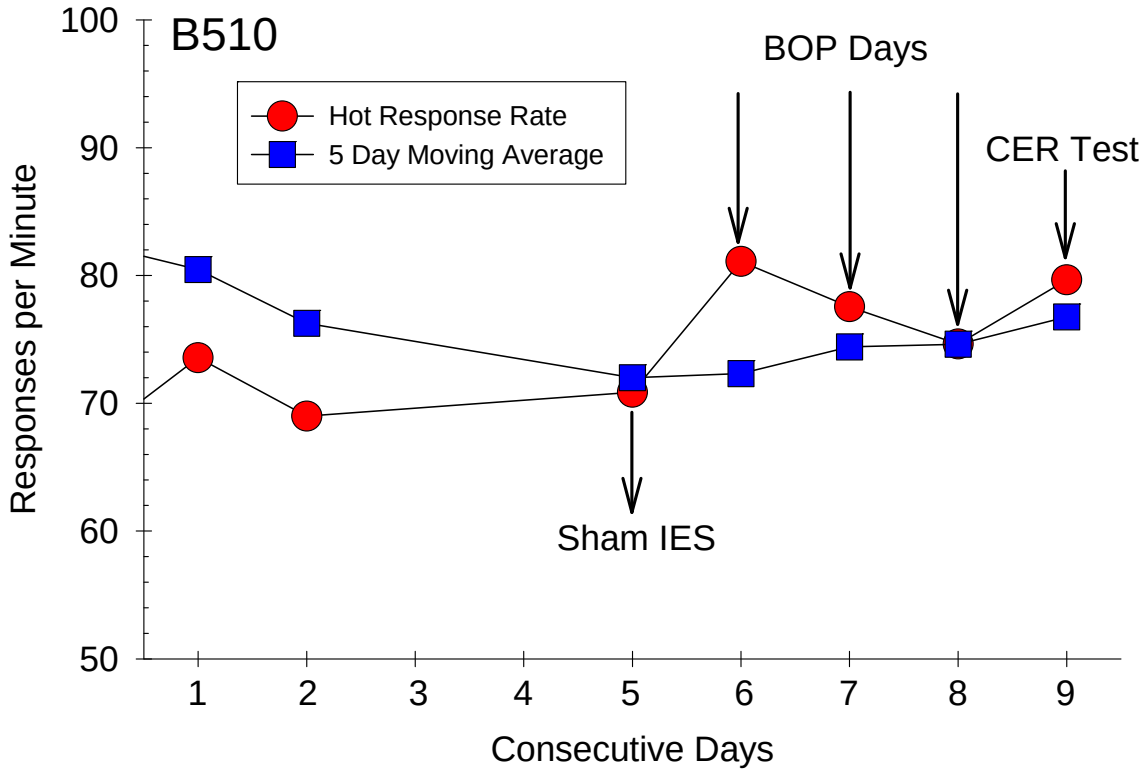


Figure 7. Performance of a single rat on the VI32 schedule. Ordinate: responses (lever presses) per minute. Abscissa: consecutive days. Circles represent session response rates on the active lever (the lever associated with food reinforcement, "Hot lever"). Squares represent the five day moving average for responding on the hot lever. BOP denotes days when blast overpressure was presented, 75kPa, ~two hours before the session. Sham IES denotes the day of presentation of the CS only and CER denotes the day that the CS was presented during the VI32 session.

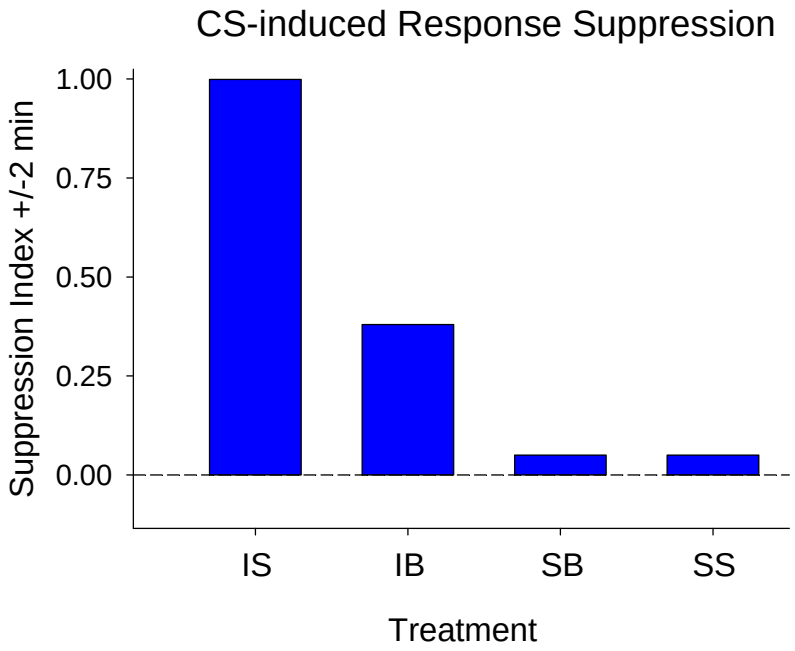


Figure 8. Response suppression (as the 2-min suppression index) to the CS during a CER test conducted 24 h after the third blast overpressure exposure and 96 h after CS+IES pairings. Treatments: IS=IES plus three sham blast overpressure exposures, IB=IES plus three blast overpressure exposures (75 kPa), SB=Sham IES plus three blast overpressure exposures (75 kPa), SS= Sham IES plus three sham blast overpressure exposures. Each bar represents data from a single rat.